

Kuru prions and sporadic Creutzfeldt–Jakob disease prions have equivalent transmission properties in transgenic and wild-type mice

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Kuru provides our principal experience of an epidemic human prion disease and primarily affected the Fore linguistic group of the Eastern Highlands of Papua New Guinea. Kuru was transmitted by the practice of consuming dead relatives as a mark of respect and mourning (transumption). To date, detailed information of the prion strain type propagated in kuru has been lacking. Here, we directly compare the transmission properties of kuru prions with sporadic, iatrogenic, and variant Creutzfeldt–Jakob disease (CJD) prions in *Prnp*-null transgenic mice expressing human prion protein and in wild-type mice. Molecular and neuropathological data from these transmissions show that kuru prions are distinct from variant CJD and have transmission properties equivalent to those of classical (sporadic) CJD prions. These findings are consistent with the hypothesis that kuru originated from chance consumption of an individual with sporadic CJD.

variant CJD | transmissible spongiform encephalopathy | iatrogenic CJD

Prion diseases are fatal neurodegenerative disorders that include Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker disease, fatal familial insomnia, kuru, and variant CJD (vCJD) in humans and bovine spongiform encephalopathy (BSE) in cattle and scrapie in sheep (1, 2). Their central feature is the posttranslational conversion of host-encoded, cellular prion protein (PrP^C) to an abnormal isoform, designated PrP^{Sc} (1, 2). This transition appears to involve only conformational change rather than covalent modification and confers PrP^{Sc} with partial resistance to proteolytic degradation and detergent insolubility (1–3). Human prion diseases are biologically unique in that the disease process can be triggered through inherited germ-line mutations in the human prion protein gene (*PRNP*), infection (by inoculation, or in some cases by dietary exposure) with prion-infected tissue, or by rare sporadic events that generate PrP^{Sc} (1–5). Substantial evidence indicates that an abnormal PrP isoform is the principal, if not the sole, component of the transmissible infectious agent, or prion (1–3).

The marked clinical heterogeneity observed in human prion diseases has yet to be explained. However, it has been clear for many years that distinct isolates, or strains, of prions can be propagated in the same host, and these are biologically recognized by distinctive clinical and pathological features on transmission to laboratory animals (2, 6). It is likely that a significant proportion of the clinicopathological heterogeneity seen in sporadic CJD and other human prion diseases relates to the propagation of distinct human prion strains. Within the framework of the protein-only hypothesis of prion propagation, distinct clinical and neuropathological phenotypes associated with prion strains are thought to be determined by the propagation of distinct PrP^{Sc} isoforms with divergent physicochemical properties (1, 2, 7–12). Genetic factors also have a role in determining prion strain selection through the coding sequence of the host prion protein gene (2, 13–15), and other unknown non-PrP genetic factors have been revealed (14, 16–18).

Kuru provides our principal experience of an epidemic human prion disease and affected the Fore linguistic group of the Eastern Highlands of Papua New Guinea and to a lesser extent neighboring groups with whom the Fore intermarried (19–23). It was the practice in these communities to engage in consumption of dead relatives as a mark of respect and mourning (transumption). Kuru was the first human prion disease shown to be transmissible, by inoculation of nonhuman primates with autopsy-derived brain tissue (24). Interest in kuru has been renewed by the appearance of vCJD, in the United Kingdom from 1995 onward (25), and the experimental confirmation that this is caused by the same prion strain as that causing BSE in cattle (9, 14, 26, 27). This has led to widespread concern that exposure to the epizootic of BSE, and subsequent transmission of vCJD prions via iatrogenic routes, poses a distinct and conceivably a severe threat to public health in the United Kingdom and other countries (4, 5, 23, 28–33). The extremely prolonged and variable incubation periods seen with prion diseases when crossing a species barrier means that it will be some years before the parameters of any human vCJD epidemic can be predicted with confidence (23, 28, 34–36). Remarkably, kuru demonstrates that incubation periods of infection with human prions can exceed 50 years (23).

Genetic susceptibility is important in both the sporadic and acquired forms of human prion disease; human PrP has a common polymorphism, with either methionine or valine present at residue 129. Approximately 38% of Europeans are homozygous for the more frequent methionine allele, 51% are heterozygous, and 11% homozygous for valine. Most sporadic CJD occurs in individuals homozygous for this polymorphism (22, 37–41). This susceptibility factor is also relevant in the acquired forms of CJD, most strikingly in vCJD; all clinical cases studied so far have been homozygous for codon 129 methionine of the PrP gene *PRNP* (4). The *PRNP* codon 129 genotype has shown a pronounced effect on kuru incubation periods and susceptibility, and most elderly survivors of the kuru epidemic are heterozygotes (22, 40). The clear survival advantage for

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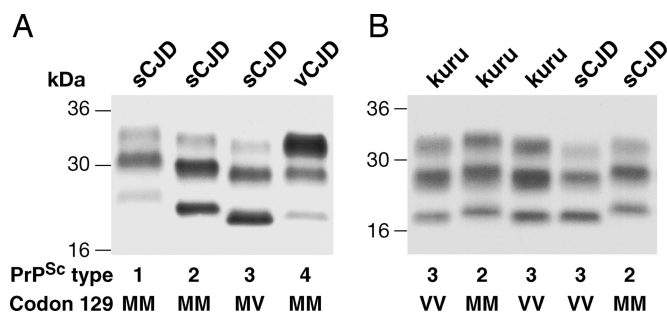


Fig. 1. PrP immunoblots of prion-diseased human brain. Immunoblots of proteinase K-digested brain homogenates from patients with human prion disease analyzed by enhanced chemiluminescence using anti-PrP monoclonal antibody 3F4 are shown. The provenance of each brain sample is designated above each lane, and the type of PrP^{Sc} detected in each sample and the *PRNP* codon 129 genotype of the patient (M, methionine; V, valine) is designated below. (A) Representative immunoblot demonstrating the London classification of human PrP^{Sc} types (9, 46). PrP^{Sc} types 1–3 are seen in the brain of classical forms of CJD [either sporadic (sCJD) or iatrogenic CJD], whereas type 4 PrP^{Sc} is uniquely seen in vCJD brain. (B) Immunoblot of the three kuru samples of the present study compared with sporadic CJD control samples of known PrP^{Sc} type.

PRNP codon 129 heterozygotes provides a powerful basis for balancing selection pressure in the Fore (22).

The origins of kuru have remained somewhat obscure. Previously unknown to the outside world, kuru deaths were recorded continuously from 1957 (42). From oral history, the first remembered kuru patient dated from the early 20th century, although the disease rapidly increased in incidence. A peak annual mortality of >2% was recorded in some Fore villages, largely of adult women and children. Some villages consequently became devoid of young women (19, 42). Over the latter half of the 20th century, kuru has progressively died out from the young Fore, consistent with the cessation of cannibalism in the late 1950s (42). However, a series of kuru patients have been examined in the last decade, all born before 1959, some with estimated incubation times of >50 years (23). In the absence of a pathogenic *PRNP* mutation associated with kuru (22, 40), the most plausible hypothesis for the origin of kuru is from chance consumption of an individual with sporadic CJD (43), which occurs worldwide with an annual incidence of ≈1–2 per million. Consistent with this hypothesis, experimental transmission rates of kuru isolates to nonhuman primates are more similar to those of classical (sporadic and iatrogenic) CJD isolates rather than inherited forms of prion disease (44). Here, we directly compare the transmission properties of kuru prions with sporadic, iatrogenic, and variant CJD prions in transgenic mice expressing human PrP and in wild-type mice. These data show that kuru prions are quite distinct from vCJD prions and have transmission properties closely similar to classical CJD prions. These findings are consistent with the contention that kuru originated from an individual with sporadic CJD.

Results

Kuru Prions Are Associated with the Same PrP^{Sc} Types That Are Seen in Classical CJD. To date, we have identified four major types of human PrP^{Sc} associated with sporadic and acquired human prion diseases that can be differentiated on immunoblots after limited proteinase K digestion of brain homogenates (9, 45, 46) (Fig. 1A). The different PrP fragment sizes seen on immunoblots after treatment with proteinase K (corresponding to amino-terminally truncated cleavage products generated from di-, mono-, or nonglycosylated PrP^{Sc}) suggest that there are several different human PrP^{Sc} conformations, referred to as molecular strain types and these can be further classified by the ratio of the three

PrP fragments seen after protease digestion. PrP^{Sc} types 1–3 are seen in classical (sporadic or iatrogenic) CJD brain, whereas type 4 PrP^{Sc} is uniquely seen in vCJD brain (9, 45, 46) [Fig. 1A and supporting information (SI) Table 1]. A simpler molecular classification (10, 11), based on subdivision into only two molecular subtypes by fragment size, is also in use, and an international consensus on human prion strain types has yet to be reached.

Polymorphism at human PrP residue 129 places constraints on the propagation of distinct human PrP^{Sc} types, and these data provide a molecular basis for this polymorphism acting as a major factor influencing both prion disease susceptibility and phenotype in humans (2, 4, 5, 22). To date, human PrP^{Sc} types 1 and 4 have been found only in humans homozygous for *PRNP* codon 129 methionine, type 3 PrP^{Sc} is seen almost exclusively in individuals with at least one valine allele, whereas type 2 PrP^{Sc} has been commonly observed in all codon 129 genotypes (9, 45, 46).

The three kuru brain samples investigated in the present study showed protease-resistant PrP fragment patterns corresponding to either the type 2 or type 3 PrP^{Sc} pattern that we have observed in sporadic and iatrogenic CJD (SI Table 1) (Fig. 1B). The glycoform ratio of protease-resistant PrP fragments in these kuru samples all showed a predominance of monoglycosylated PrP similar to the PrP glycoform ratios seen in classical CJD (Fig. 1B) rather than the distinctive PrP glycoform ratios seen in either vCJD or inherited prion disease caused by *PRNP* point mutations (46–48). These data are consistent with previous findings showing close similarity of PrP^{Sc} types in kuru and classical CJD (49, 50).

Kuru Prions and Classical CJD Prions Have the Same Transmission Rates in Transgenic or Wild-Type Mice. Our series of transmitted samples from patients with neuropathologically confirmed sporadic, iatrogenic, and variant CJD are shown in SI Table 1. Transgenic mice expressing human PrP 129 valine, but not mouse PrP (129VV Tg152 mice) lack a transmission barrier to classical (sporadic and iatrogenic) CJD prions, regardless of the codon 129 genotype of the inoculum (9, 26, 51) (SI Table 1). These transmissions are characterized by 100% attack rates of prion infection producing clinical prion disease with similar short incubation periods of ≈200 days (9, 26, 51) (SI Table 1). In isolates that have been examined, no fall in mean incubation period is seen after secondary passage in further 129VV Tg152 mice indicative of the lack of a transmission barrier (ref. 51; unpublished data). In contrast, primary challenge of 129VV Tg152 mice with vCJD prions is characterized by a substantial transmission barrier with only ≈50% of inoculated mice becoming infected (15, 26) (SI Table 1). Affected vCJD-inoculated Tg152 mice propagate a novel prion strain associated with type 5 PrP^{Sc} (15, 26) that fails to adapt after secondary passage in further 129VV Tg152 mice (15). In wild-type FVB/NHsd mice [genotype *Prnp*⁰ (52)], the relative transmission efficiencies of classical and variant CJD prions are quite different (9, 15, 26, 51) (SI Table 1). Classical CJD prions transmit infection to FVB/NHsd mice only occasionally, at long and variable incubation periods, whereas vCJD prions transmit infection far more efficiently, although incubation periods are prolonged (14, 15, 26) (SI Table 1). Consistent with the dramatic difference in the observed transmission rates of sporadic CJD and vCJD prion strains, highly distinct PrP^{Sc} types are propagated in affected vCJD- or sporadic CJD-inoculated FVB mice (26, 53) (Fig. 2A).

In the present study, kuru-inoculated 129VV Tg152 mice and FVB/NHsd mice showed transmission rates closely similar to those of classical CJD prions rather than those of vCJD prions (SI Table 1). In 129VV Tg152 mice, all three kuru isolates produced 100% attack rates of prion infection causing clinical prion disease with closely similar mean incubation periods of

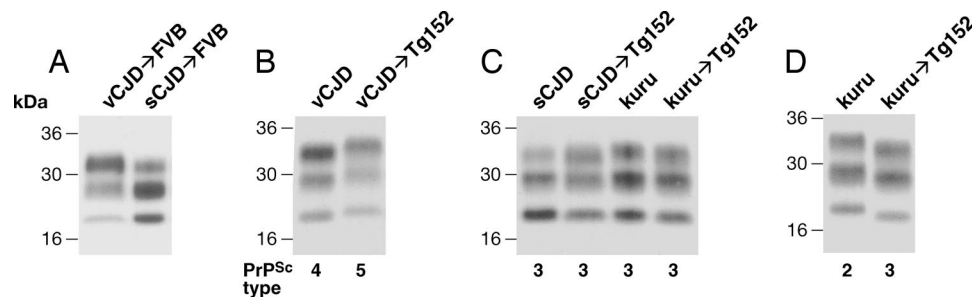


Fig. 2. Molecular strain typing of human prion transmissions to mice. Immunoblots of proteinase K-digested brain homogenates from wild-type mice, human patients, or transgenic mice analyzed by enhanced chemiluminescence with anti-PrP monoclonal antibodies ICSM35 (A) or 3F4 (B–D) are shown. The provenance of each brain sample is designated above each lane, and the type of human PrP^{Sc} detected in each sample (B–D) is designated below. (A) Transmission of vCJD prions (I344) and sporadic CJD prions (I764) to FVB/NHsd mice. (B) Transmission of vCJD prions (I348) to 129VV Tg152 mice. (C) Transmission of sporadic CJD prions with type 3 PrP^{Sc} (I4855) and kuru prions with type 3 PrP^{Sc} (I516) to 129VV Tg152 mice. (D) Transmission of kuru prions with type 2 PrP^{Sc} (I518) to 129VV Tg152 mice revealing a change in molecular strain type.

≈200 days (SI Table 1). In sharp contrast, no evidence for prion infection was seen in kuru-inoculated FVB/NHsd mice after prolonged observation periods (SI Table 1).

Transgenic Mice Inoculated with Kuru Prions and Classical CJD Prions Show the Same Molecular and Neuropathological Phenotype. Transgenic modeling of classical CJD and vCJD prion infection in mice expressing human PrP with either 129 valine or methionine (9, 14, 15, 26, 51, 54) indicates that this polymorphism directly dictates the propagation of distinct PrP^{Sc} conformers, consistent with a conformational selection model of prion transmission barriers (2, 13, 15, 28). Human PrP^{Sc} types 1 and 4 that are seen only in *PRNP* codon 129 methionine homozygous patients do not replicate faithfully in 129VV Tg152 mice. Type 1 PrP^{Sc} converts to a PrP^{Sc} type with maintained glycoform ratio but a type 2 fragment size (9), and type 4 PrP^{Sc} converts to type 5 PrP^{Sc} with the same glycoform ratio as type 4 PrP^{Sc} but a type 2 PrP^{Sc}-like fragment size (15, 26) (Fig. 2B). In contrast, PrP^{Sc} types 2 and 3 appear to propagate faithfully in 129VV Tg152 mice with no apparent change in proteolytic fragment size (9).

Consistent with these observations, and in agreement with the similarities of both PrP^{Sc} type and the transmission properties of classical CJD and kuru prions, type 3 PrP^{Sc} from kuru brain isolates propagated faithfully in 129VV Tg152 mice (Figs. 2C and 3). Interestingly, however, all affected 129VV Tg152 mice inoculated with type 2 PrP^{Sc} from the *PRNP* codon 129 methionine homozygous kuru patient also showed propagation of type 3 PrP^{Sc} in brain, indicating a switch in molecular strain type (Figs. 2D and 3). To date, we have examined the transmission properties of only a single case of classical CJD from a codon 129 methionine homozygous patient with type 2 PrP^{Sc} in brain (SI Table 1). This isolate showed apparently faithful replication of type 2-like PrP^{Sc} fragment size in 129VV Tg152 mice (9). The observed variance in the behavior of the classical CJD prions and kuru prions associated with type 2 PrP^{Sc} and methionine at codon 129 may reflect strain-specific differences between subtypes of sporadic CJD-like prions; however, further transmissions of classical CJD isolates with this molecular strain type will now be required to investigate this directly.

Both kuru-inoculated 129VV Tg152 mice and sporadic CJD-inoculated 129VV Tg152 mice propagating type 3 PrP^{Sc} showed essentially the same neuropathological changes (Fig. 3). In these mice, spongiform change was mainly restricted to the thalamus and hypothalamus. Abnormal PrP deposition had the same distribution as spongiform change characterized by a predominantly diffuse pattern of PrP staining throughout the thalamus and hypothalamus occasionally accompanied by small PrP plaques in the corpus callosum (Fig. 3). In sporadic CJD-inoculated 129VV Tg152 mice propagating type 3 PrP^{Sc}, diffuse

abnormal PrP staining was also occasionally observed in the brainstem (Fig. 3). In sharp contrast, vCJD-inoculated 129VV Tg152 mice propagating type 5 PrP^{Sc} show a very different pattern of abnormal PrP deposition. In clinically affected mice with type 5 PrP^{Sc}, a pericellular pattern of PrP immunostaining was originally detected that was difficult to distinguish from mock-inoculated controls because of a high level of PrP overexpression (26). However, after secondary passage, although no clinical prion disease is observed, aged subclinically infected 129VV Tg152 mice with type 5 PrP^{Sc} have large, nonflorid, PrP plaques in the corpus callosum with an absence of diffuse PrP deposition in other brain areas (15). In the present study, we have now also observed this distinct pattern of PrP pathology in aged subclinically infected 129VV Tg152 mice after primary challenge with vCJD prions (Fig. 3 and SI Table 1). These collective data establish that the neuropathology of kuru- and sporadic CJD-inoculated 129VV Tg152 mice are closely similar and are quite distinct from the neuropathology seen in the same line of mice after primary challenge or secondary passage of vCJD prions.

Discussion

In the present study, we have compared the transmission properties of kuru prions with sporadic, iatrogenic, and variant CJD prions in transgenic mice expressing human PrP valine 129 and in wild-type mice. These data establish that kuru prions have transmission properties equivalent to those of classical CJD prions and are distinct from vCJD prions. In agreement with this finding, the molecular strain types of PrP^{Sc} seen in kuru brain correspond to those seen in classical CJD rather than the distinct PrP^{Sc} types seen in vCJD or inherited prion disease. These collective data establish that kuru and classical CJD prions have closely similar prion strain properties and are consistent with the hypothesis that kuru originated from chance consumption of an individual with sporadic CJD (43).

Human prion diseases with distinct etiologies are associated with a range of clinical presentations that are now seen as clinicopathological syndromes rather than individual disease entities (4, 55, 56). The central clinical feature of kuru is progressive cerebellar ataxia, and, in sharp contrast to sporadic CJD, dementia is a late and less prominent feature. A prodrome and three clinical stages consisting of an ambulatory stage, a sedentary stage, and a tertiary stage have been described in ref. 20. In this regard, the natural history of kuru is more reminiscent of vCJD in which early symptoms of distal sensory disturbance, joint pains, and psychiatric and behavioral changes are common before forthright dementia (4, 23, 25, 28). Despite these similarities in early clinical presentation, the molecular and neuropathological features of kuru are distinct from vCJD. In contrast to the occurrence of abundant florid PrP plaques that are the

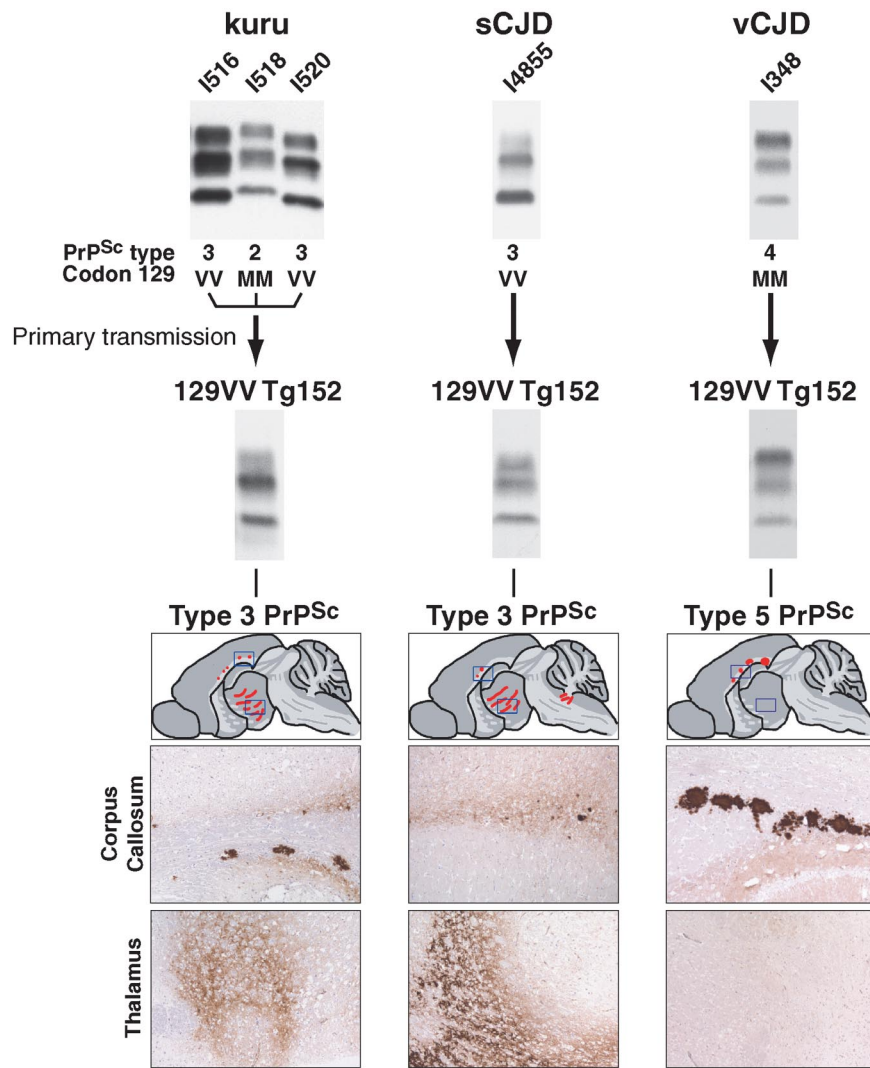


Fig. 3. Neuropathological analysis of transgenic mouse brain. Equivalent patterns of neuropathology are seen in 129VV Tg152 mice that propagate type 3 PrP^{Sc} after primary transmission of kuru prions or sporadic CJD prions that are distinct from 129VV Tg152 mice that propagate type 5 PrP^{Sc} after primary transmission of vCJD prions. Sketches represent the regional distribution of abnormal PrP deposition in transgenic mouse brain: diffuse synaptic PrP deposition (bars) and PrP plaques (circles). The bottom images show PrP immunohistochemistry with ICSM 35 (from the areas delineated by the blue boxes in the sketches) demonstrating nonflorid PrP plaques in the corpus callosum and diffuse synaptic PrP deposition in the thalamus. (Scale bar, bottom images: 100 μ m.)

neuropathological hallmark of vCJD (25, 57, 58), the neuropathological changes seen in kuru lie within the spectrum of those seen in sporadic CJD. Unicentric PrP plaques, however, are unusually prominent and widespread (58, 59). This pattern of neuropathology most closely resembles a relatively rare subtype of sporadic CJD associated with long clinical duration and *PRNP* codon 129 heterozygosity in which kuru-type PrP plaques are also observed (10, 11, 46).

Although PrP plaques are not a prominent feature of the most common subtypes of sporadic CJD, where diffuse synaptic PrP deposition predominates (10, 11, 46, 60), kuru-type plaques are a notable feature of iatrogenic CJD resulting from peripheral inoculation, most conspicuously after cadaveric pituitary-derived hormone exposure (61). This form of iatrogenic CJD also typically presents with a progressive cerebellar syndrome reminiscent of kuru, whereas cases of iatrogenic CJD arising from intracerebral or ocular inoculation usually manifest clinically as sporadic CJD, with a rapidly progressive dementia (62–65). These observations suggest that cerebellar onset and subsequent neuropathological changes may be determined in

part by a peripheral route of exposure. The similarity of prion strain type in kuru and sporadic CJD demonstrated here now clearly suggests that peripheral routes of infection (predominantly dietary), rather than prion strain type, may be an important determinant of the clinicopathological phenotype of kuru. In this regard, the etiology of sporadic CJD remains unclear, although its remarkably uniform worldwide incidence and apparently random distribution suggest involvement of a stochastic process such as somatic *PRNP* mutation (2, 48, 66, 67). It is thus possible that part of the phenotypic and neuropathological heterogeneity seen in sporadic CJD could be related to peripheral versus central initiation of prion replication.

Aside from route of exposure, additional factors may also influence the neuropathology and clinical features of kuru. A number of genetic modifiers of prion disease have been mapped in mice (16, 17). Although the genes responsible for mouse incubation time quantitative trait loci have not yet been identified, orthologous human genes are likely to be globally polymorphic with significant differences within and between Europe and the Fore. Furthermore, age is an important determinant of

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